PRESERVATION OF ANIMAL GERM PLASM - AN OVERVIEW

B. BERESKIN
AGRICULTURAL RESEARCH SERVICE
U.S. DEPARTMENT OF AGRICULTURE
BELTSVILLE, MARYLAND

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B. Bereskin [1,2/]

Agricultural Research Service

U.S. Department of Agriculture, Beltsville, Maryland 20705

EARLY AND RECENT HISTORY

Much of the early history of the earth remains shrouded in uncertainty. However, geological evidence has clearly shown that countless forms of animal life have inhabited the earth down through the many eons of time. Long before the emergence of man, the successive evolution and subsequent extinction of innumerable species of animals was the general rule. Examples are the many forms of dinosaurs which are thought to have expired some 65 million years ago, after having been the dominant life form for a very long period. Violent environmental changes are credited with producing those extinctions.

In subsequent ages, various mammalian species evolved and spread over the earth. Man is thought to have come on the scene some 2 million years ago. Fossil remains indicate that many of the early mammalian species became extinct during the pleistocene period; including the mammouth, mastadon, woolly rhinocerous, saber-toothed tiger and others. That age was marked by periods of major glaciation and extended to about 30,000 years ago, when the last great glaciers are thought to have retreated.

Since that period man probably first domesticated cattle, sheep, goats, swine, horses, and other mammals, as well as various species of fowl. This was long before man's recorded history, which dates back little more than 6,000 years.

1/ Non-ruminant Animal Nutrition Laboratory, Nutrition Institute.

2/ Adapted from a paper presented at the BARC Workshop on Germ Plasm Preservation, Beltsville, Maryland, October 27-28, 1976.

Numerous local races or breeds of the major domesticated species were developed by man as he settled in the far corners of the world and engaged in agricultural pursuits. Great diversity in the features and performance among the many local breeds gradually evolved over the centuries, each breed suited to the special needs of man in that area and well adapted to the local environment.

Impact of Agricultural Sciences

This process of local diversity continued largely unchanged until the present century. Then came the development of genetics, reproductive physiology and other agricultural sciences. Concurrently, human populations increased rapidly, requiring greatly expanded food production. All these factors stimulated dramatic improvements in the productivity of a relatively small number of breeds or strains of the common livestock species. In turn, these improved strains began to replace local breeds around the world, leading to the extinction or near extinction of many of them. As a result, in recent years we have seen an increased concern about preserving the threatened domesticated stocks.

Scientific studies of agricultural practices have also stimulated interest in other forms of animal life, such as microorganisms and various species of insects which directly or indirectly affect agricultural production. The need to adequately preserve and manage the life cycles of these organisms is now fully recognized.

Also, extensive scientific research on laboratory animals and other animal species, usually with direct applications to human welfare, has resulted in major efforts to preserve the affected species for such purposes.

Wildlife

We are witnessing the extinction or near extinction of numerous species of wildlife in this century. This resulted mainly from extensive destruction of natural habitat or the practice of overharvest by man. Here too, major efforts have recently been directed at protecting those endangered and threatened species.

Thus, we see that efforts in germ plasm preservation are being directed along a very broad front, involving numerous species of animals. These efforts have generally followed two main courses: the natural propogation of whole organisms or the artificial manipulation of life cycles of either the whole organism or components thereof.

Let us now briefly examine several examples of man's efforts to preserve animal germ plasm. First, let us look at preservation by natural propogation.

PRESERVATION BY NATURAL PROPOGATION

Jackson Laboratory

The Jackson Laboratory at Bar Harbor, Maine, is generally recognized as the world's pioneer and leader in such efforts. Since its founding in 1929, the Laboratory has investigated, catagorized, developed, maintained and disseminated largely uniform genetic stocks of laboratory mice for research purposes around the world [Green (2)[3/]]. Their present inventory includes some 60 highly inbred lines, 7 F_1 crosses and scores of mutation crosses. Various stocks of rabbits are similarly maintained and disseminated for experimental purposes [Fox (1)].

Swine Gene Pool

A completely different approach to preserving animal germ plasm was taken by Dr. Lavon Sumption at the University of Nebraska [Zimmerman and Cunningham (8)]. Instead of maintaining separate breeds or inbred lines as the Jackson Laboratory has done, he developed over a period of years a gene pool strain of 14 different swine breds, starting in 1958. These included the popular American breeds, along with some European and lesser known breeds, even including the European wild pig.

Final incorporation of the breeds was in 1965. After that the strain was separated into 2 lines and selection experiments, dealing mainly with reproductive performance, were initiated. At last report, one line is still being maintained. This is an example of preserving genes rather than separate breeds and may have practical value in some situations.

[3/] Underscored numbers in parentheses refer to Literatured Cited, p. 17.

Registry of Poultry Stocks

Interest in preserving poultry germ plasm dates to the early 1900's. The USDA, in 1962, initiated a survey to establish a registry of poultry germ plasm in the United States. However, none was compiled. In 1967, Dr. Roy Crawford of Canada published the world's first catalogue of poultry stocks, describing those maintained at reasearch and teaching institutions in Canada.

In 1972, Dr. Ralph Somes of the University of Connecticut published the first registry of chicken genetic stocks in the United States. It included the description and location of 87 specialized lines and strains, the inheritance and linkage information on 103 mutant traits, an up-to-date chromosome map, the location of 260 breeds and varieties and a registry of breeders and suppliers.

Dr. Somes (6) published an enlarged edition in 1975, with information on U.S. chicken stocks, plus similar data on Canadian stocks, the Japanese quail and the turkey. It is generally acknowledged that these registries, along with those from the Jackson Laboratory, provide models for similar efforts needed in other species of domesticated animals. This kind of information is essential before programs of preservation can be initiated with any hope of success.

Endangered Livestock

I believe it is safe to say that no species of the major farm animals in the United States faces imminent extinction over the whole country at the present time. These include cattle, swine, sheep, goats, horses and various species of domestic fowl. However, the prospects are real for the local extinction of a number of breeds or strains within most of those species and the status of other breeds is becoming increasingly precarious.

Thus, in chickens, despite the fine registries, only very small collections of many strains and breeds are maintained by State and Federal experiment stations or by individuals, groups or some commercial firms. Many of these stocks are being allowed to become extinct when they have served the immediate needs of the investigator or firm.

In beef cattle, the Galloway breed faces imminent extinction in the United States, with only 4 active breeders registering 19 head in 1975. In dairy cattle, the Dutch Belted breed had only 26 active breeders, registering 191 head in 1975. Only 385 head of Hereford hogs were registered in 1975, down from 625 in 1967. Only 145 head of Lincoln sheep were recorded in 1975, down from 358 in 1967. Also, the Percheron and Belgian draft horse breeds, along with the donkey, a separate species, have shown precipitous declines in population over the past 30 years in this country.

Animal Preservation in Great Britain

It is well known that many of our present breeds of American farm animals were developed and improved in the British Isles during the 18th and 19th centuries. With their rich tradition and history of livestock improvement, it is no wonder that the British people have taken a keen interest in preserving many of their native breeds. But the direction of their efforts is unique.

In 1970 [Mason (4)], an extensive survey was made on the numbers and location of all native breeds thought to be facing extinction. In 1971, a convention of interested persons was held, at which time the Rare Breeds Survival Trust was established. It would be entirely controlled and directed by the private sector and financed by the general public and charitable groups.

Its primary objectives were to keep an accurate and up-to-date account of the status of the various threatened breeds in the country; to educate the public on the need to preserve irreplaceable genetic resources; and to encourage the setting up of so-called "farm parks" by individuals or groups. These would contain small herds or flocks of rare breeds, to be viewed by the public for a fee. This would help defray the cost of maintenance and provide some revenue for the Trust. To date the Trust has been notably successful in raising money and in establishing farm parks and refuges for threatened breeds. It has even purchased a Scottish island where the Orkney sheep, which eat seaweed, will be protected.

This British effort has provided the best and perhaps only example to date of how a large burden of preservation with potentially important biological consequences can be met by income from a leisure activity having educational merit. However, despite current interest and some success, the long-range prospects for this approach remain uncertain. Nevertheless, there would seem to be a place for this approach in the United States and elsewhere.

In France and in several other developed countries, a situation similar to that in Great Britain prevails, with losses having already occurred or in prospect for the near future. In most of these countries, organized efforts to preserve vanishing local breeds or strains have not been initiated or have met with little success to date.

UNITED NATIONS EFFORTS

The United Nations (U.N.) Food and Agriculture Organization (FAO) first became interested in the problem in the 1950's. Several exploratory meetings were held to learn more about the situation and discuss possible approaches to take. In the 1960's and early 1970's more conferences were held, dealing with specific issues or species.

The Stockholm Conference on the Environment in 1972 authorized the U.N. Environmental Program to work closely with FAO in expanded efforts to protect and conserve rare or endangered species, including wildlife and domestic animals. Various cooperative projects were initiated.

Survey of European Cattle

One such project was a survey of endangered cattle breeds in Europe and the Mediterranean region, with 32 countries supplying information [Lauvergne (3)]. It was found that 115 indigenous breeds of cattle were on the verge of extinction, with only 30 others holding their own. The dis-appearance of dairy breeds was very advanced, with only 2 or 3 likely to survive in the near future. The triple-purpose breeds--milk, meat and work, faced an equally bleak future.

The survey also identified the major causes of these drastic changes in the status of many breeds. These included the search for higher yielding breeds, primarily for dairy purposes; the availability of artificial insemination with so-called improved breeds; cattle no longer used for draught purposes; changing farming structure, with

small local breeders retiring or dying and their breeds being discarded; and government efforts to promote intensified production to feed increasing human populations by replacing local breeds with imported improved breeds. The researchers learned that not all the changes produced the desired results, as for example, where local cattle are better adapted to the local environment.

Other U.N. Efforts

The U.N. agencies also sponsored studies of breed dynamics on the islands of Corsica and Sardinia. The purpose was to learn of possible ways to maintain local indigenous breeds in an isolated environment in the face of importations of so-called improved breeds. The conclusions were not optimistic. The agencies also sponsored the first "World Congress on Genetics and Livestock" in Madrid in 1974. This featured speeches and panel discussions by recognized authorities from around the world on the conservation of animal genetic resources.

Thus, we have seen that U.N. agencies have sponsored various useful conferences and studies on the problems of preserving germ plasm of domestic animals, but few definitive results have accrued to date. Prospects for effective U.N. efforts in the future appear mixed at this time.

ENDANGERED WILDLIFE

In general, the world's wildlife are now in a much more precarious position than domesticated animals, in terms of species survival [Strohm (7)]. For example, in the United States and Puerto Rico alone, only two species of mammals and three of birds are thought to have become extinct from 1600 to 1850. Since 1850, 17 species of mammals, 28 species of birds, and 12 species of fish are considered to have become extinct. These include the Eastern Elk, Buffalo Wolf, Atlantic Gray Whale, Carolina Parakeet and the Passenger Pigeon.

The latest list of endangered species for the U.S. and adjacent waters contains 109 mammals, birds, and other animal forms. These include eight species of whales, the Eastern Timber Wolf, California Condor, Whooping Crane, and Southern Bald Eagle. In addition 188 species are listed as threatened.

This critical situation has led to major new efforts to protect endangered wildlife around the world. However, the prospects continue to deteriorate for many species. More wildlife refuges and wilderness areas on land and stricter international controls on the harvest of marine mammals offer some hope. However, it will probably take a much wider acceptance by man of an enlightened conservation ethic before these and other necessary measures are fully implemented.

MANIPULATION OF LIFE CYCLES

Let us now turn to the preservation of animal germ plasm by artificial manipulation of life cycles of either the whole organism or certain of its components.

Artificial Insemination (A.I.)

The increase in A.I. since about 1930 paralleled improved techniques in semen preservation [Neville (5)]. The most dramatic results have occurred with cattle, primarily dairy cattle. Prior to 1950, only non-frozen semen was used. Dr. Polge in England in the late 1940's discovered that glycerol afforded protection to sperm during the stress of freezing to ultra-low temperatures. His discovery is credited with opening the door for the remarkable subsequent development of cryobiological techniques for the long-term preservation of a variety of cell types.

Most A.I. with cattle around the world now uses frozen semen. However, artificial insemination of swine has not yet proven economically feasible on a large scale in the United States. While significant advances in the application of A.I. to swine have been made at Beltsville and other research centers, some problems still remain with freezing procedures and insemination techniques. The ready availability of performance tested boars also continues to restrict the use of A.I. in swine.

For sheep and goats, frozen semen has been used in conjunction with estrus control in A.I. programs. However, the results have not been consistent enough to warrant commercial application on a wide scale. Further research is needed.

Although A.I. is standard practice with turkeys, only fresh semen has proven feasible. The same apparently is true for chickens. Glycerol, the common cryoprotective agent, has been shown to have a

contraceptive action in fowl semen even at a level of 1%. Thus new techniques for freezing apparently are needed.

Other domestic species, including the dog, also are having variable degrees of difficulty with applications of cryobiological techniques to breeding problems, but progress, although slow, is being made.

Humans.--The first report of successful use of frozen semen in humans was in 1953. A 1972 report indicated that 400 live births had resulted from use of frozen human semen. In one case it had been stored for 10 years. The report also noted that the DNA in the sperm remained constant during 6 years of storage, thereby allaying fears of genetic changes due to freezing.

Efforts with wildlife.--Fresh semen has been used successfully for artificial insemination of several captive avian wildlife species, including the endangered Whooping Crane at the nearby Patuxent Wildlife Research Center. However, use of frozen semen has not been successful, as it faces the same problems plaguing frozen poultry semen. Intensified research efforts are continuing on the preservation of semen and fertilized ova of avian species with application to endangered wildlife.

Expanded efforts also are under way by the larger zoos, including the National Zoo in Washington, to obtain semen by electroejaculation methods from captive wildlife such as the large cats, subhuman primates, ungulates and other rare or endangered species for purposes of propogation in captivity.

Insects and Other Animals

The nematode DD-136 with its associated bacteria is capable of attacking and killing many insect pests. Stock cultures of the nematode-bacteria complex can be preserved for long periods in specially prepared agar slants. For example, 27-year-old preparations are still intact at Beltsville. Storage of various stages of the nematode in stock solutions or in glycerine suspensions for shorter periods of time is also feasible.

Insect tissues of various kinds have been preserved successfully in liquid nitrogen. These include blood cells, ovarian somatic tissue and embryos. To date, this method has been used mainly to study pathogens in insect pests, such as moths, butterflies, and mosquitos. Other insects in which preservation studies are being conducted for various purposes include the honey bee, codling moth, gypsy moth, screw worm, corn borer and fruit fly.

Microorganisms.--These include protozoa which have been maintained since antiquity for various purposes in extracts, suspensions, mashes and mixes, as well as in host animals and plants. Modern techniques such as freezing have allowed microbiologists to preserve intact organisms for extended periods and thereby avoid costly repetitious transfers in cultures. This also provides for better control and evaluation of experimental material.

Embryo Storage

As noted previously, the discovery that semen could be stored in a frozen state for prolonged periods with certain protective agents proved to be a major breakthrough in biology and has had a tremendous impact on the preservation of animal germ plasm. In 1972, two English scientists recorded a similar breakthrough by their remarkable discovery that a bovine embryo which had been stored in liquid nitrogen for 6 days before being transplanted back into a cow resulted in the birth of a normal healthy calf.

If proven feasible, the possibilities arising from the storage of embryos followed by successful re-transplants are overwhelming. These techniques, along with widespread application of frozen semen, promise to revolutionize the ideas and efforts in germ plasm preservation in the coming years.

OBJECTIVES AND NEEDS

The main reasons for preserving germ plasm can be divided into two major categories, as follows:

1. To assure survival of species or desirable breeds or strains facing extinction.
2. To allow more efficient control and manipulation of germ plasm when survival of a species, breed or strain is not a factor or of concern. This can be further subdivided as follows:
    a. To facilitate efficient utilization of available gentic resources for improvement of economic traits.
    b. To provide a stabilized population to serve as a control, bench mark or reference point for assessing treatment effects in experimental studies.

c. To allow, at lower cost than older methods, the more efficient control of life cycles in various organisms to be used for experimental and applied purposes.

Preserving Domestic Animals

Formidable problems are involved in preserving endangered domestic stocks. For example, the ability or even the need to preserve all present breeds and strains is seriously questioned. Thus, I.L. Mason, FAO Animal Breeding Officer has stated, "Preservation without utilization is doomed to failure." He recommends that local breeds able to perform well in difficult habitats such as sparse vegetation, mountainous terrain or the tropics should be utilized for their agricultural potential. Breeds that demonstrate hybrid vigor on crossing with improved or exotic breeds also merit preservation. Genetically unique breeds should be retained for scientific studies of their genetics, evolution and biochemistry. Also, says Mason, aesthetically attractive and historically important breeds should be maintained in parks and preserves for their educational and cultural values.

The most urgent need is for more information in order to decide which treatened breeds merit preservation and how best to proceed. Here is where an international agency such as FAO can play a much greater role, by promoting and coordinating efforts around the world, including the United States.

The critical issues reduce to: Who is to initiate any program of preservation? Who is to decide what to save? Who is to formulate the plans and direct the efforts? Who will pay for it? On the whole, these questions have largely remained unanswered to this day.

Preserving Wildlife

On the question of wildlife, surely man does not want to knowingly cause the extinction of any more wildlife species. To prevent this, it generally is acknowledged that man will need to set aside and protect suitable and adequate habitat for wildlife and adjust his management practices to assure the survival of biologically viable populations. Such actions may conflict with the special interests of man in some

regions of the world. Thus solutions need to be developed that allow for the mutual survival and coexistence of man, his livestock, and his wildlife heritage on our common home, the earth.

Cryobiology

Finally, where manipulation, not extinction, of germ plasm is of primary concern, the critical need now appears to be intensified research efforts in the appropriate disciplines of biology and technology. Different methods of preservation will of course need to be examined and improved. However, most observers now agree that the primary emphasis of such research should relate to cryobiology and its many applications. Cryobiology now appears to offer the best and perhaps only hope in the near and intermediate future periods to successfully preserve animal germ plasm for most purposes.

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